On Page 26, Claim 4, amend and add as follows:

A method for preparation of the compound according to claim 3, comprising the step of esterifying ~~or phosphoric esterifying~~ the second lead compound 12-β-thiocyano-13-α-hydroxy triptolide with succinic anhydride to obtain the compound of formula II, or the step of phosphoric esterifying the second lead compound 12-β-thiocyano-13-α-hydroxy triptolide with $POCl_3$, PCl_3, phosphonate halide, phosphate, phosphite halide, or phosphite to obtain the compound of formula II.

AMENDMENTS TO THE SPECIFICATION

On Page 4, line 3, amend to read: **wherein R_1 is H, alkyl having 1-4 carbon atom(s), -C(=O)$(CH_2)_n CO_2$H-, wherein n is an integer of 1-4; or ;**

On Page 4, line 11 of the PCT publication, *Succinic anhydride* should be replaced with **4-Oxo-butyric acid.**

On Page 5, line 11, the following paragraphs should be inserted after the chemical structure located on the top of the page:

The present invention provides a method for preparation of the compound of formula I, comprising the step of reacting the first lead compound triptolide with $POCl_3$, PCl_3 or other phosphonate halide, phosphate, phosphite halide, phosphite.

The present invention provides a method for preparation of the compound of formula II, comprising the step of esterifying the second lead compound 12-β-thiocyano-13-α-hydroxy triptolide with succinic anhydride to obtain the compound of formula II, or the step of phosphoric esterifying the second lead compound 12-β-thiocyano-13-α-hydroxy triptolide with $POCl_3$, PCl_3, phosphonate halide, phosphate, phosphite halide, or phosphite to obtain the compound of formula II.

The present invention provides a method for preparation of the compound of formula IIIa and IIIb, comprising the step of reacting the second lead compound 12-β-thiocyano-13-α-hydroxy triptolide with $POCl_3$ or other phosphonate halide, phosphate, phosphite and the like, or the step of reacting the first lead compound triptolide with $POCl_3$ or other phosphonate halide, phosphite halide and the like.

The present invention provides Use of the compounds of the present invention in the preparation of medicament as immunosuppressive agent or anti-inflammatory agent. In an embodiment, the immunosuppressive agent or anti-inflammatory agent is used in the

treatment of diseases associated with growth of lymphocytes T and B cells; production of cytokines such as IL-1, IL-2, IL-6, and iNOS; and production of Cox-2. In another embodiment, the diseases are autoimmune deficiency diseases and inflammatory diseases. In another embodiment, the diseases are selected from the group consisted of rheumatoid arthritis, asthma, systemic lupus erythematosus, psoriasis, multiple sclerosis, atherosclerosis, type I diabetes, and nephritis.

Page 10, line 36, please amend the line to read:

73.5 (C-14, d, J=22.8Hz), 123.2(3-C), 162.5(4-C), 173.2(20-C), 63.5, 64.5, 64.6(8-C, 9-C, 13-C).

AMENDMENT TO THE DRAWINGS

Figure1, and Figure 2, please amend all **"μg" to "μM"**.

REMARKS

Attached are the replacement pages for the above amendments.

Any additional fees required in connection with this communication which are not specifically provided for herewith are authorized to be charged to the Deposit Account No. 50-2638 in the name of Greenberg Traurig LLP. Any overpayments are also authorized to be credited to this account.

Respectfully submitted,

Date: 18 March 2005

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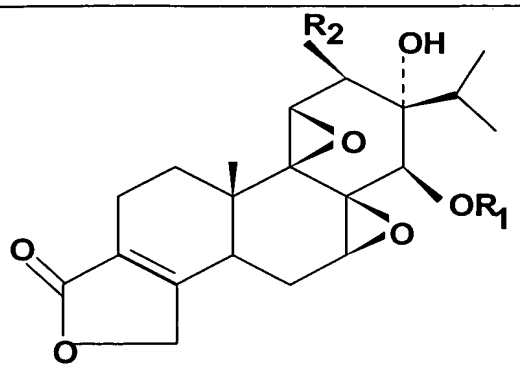


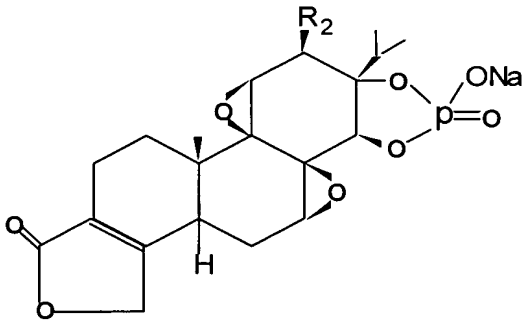
II

wherein R_1 is H, alkyl having 1-4 carbon atom(s), -Ac, -C(=O)$(CH_2)_n$$CO_2$H (n is an integer of 1-4), phosphate (), or phosphite ( ), wherein X_1 and X_2 are Na, K, or NH_4; and R_2 is H, -SCN, -Cl or -Br.

Preferably, R_1 is phosphate ( ), or

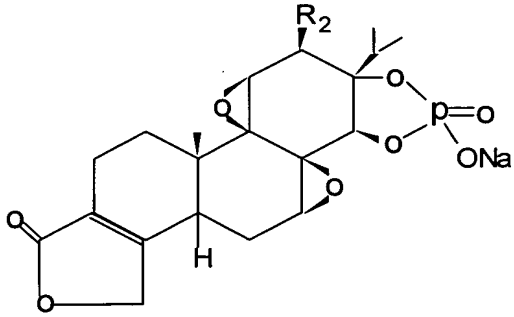
4-Oxo-butyric acid~~succinic anhydride~~ [-C(=O)$(CH_2)_4$$CO_2$H], in which X_1 and X_2 are Na, R_2 is -SCN.

The third type of novel derivatives of triptolide is as follow:



IIIa

or

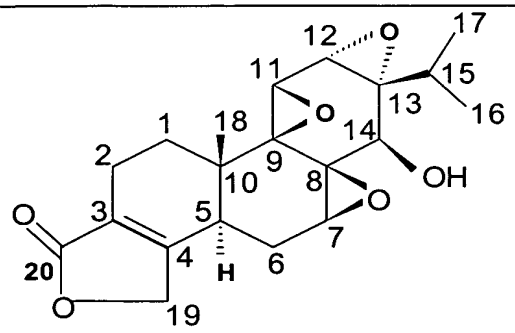


IIIb

wherein R_2 is H, -SCN, -Cl or -Br.

Another objective of the present invention is to provide the synthetic methods of these novel highly water-soluble derivatives of triptolide, and more particularly, the synthetic methods of preparing above three types of water-soluble derivatives of triptolide from the following two lead compounds (see Example 1-7 for details).

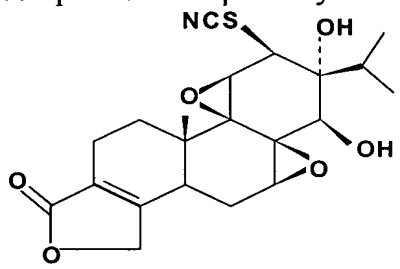
The first lead compound is triptolide:



Triptolide(T)

US Patent 5,962,516 discloses a method for the preparation of 14-succinyl triptolide and sodium salt thereof from triptolide. 14-β-phosphate bisodium triptolide produced by phosphorylation according to the present invention, however, has higher water solubility.

The second lead compound is 12-β-thiocyano-13-α-hydroxy triptolide:



12-βthiocyano-13-α-hydroxy triptolide (T-SCN)

The present invention provides a method for preparation of the compound of formula I, comprising the step of reacting the first lead compound triptolide with $POCl_3$, PCl_3 or other phosphonate halide, phosphate, phosphite halide, phosphite.

The present invention provides a method for preparation of the compound of formula II, comprising the step of esterifying the second lead compound 12-β-thiocyano-13-α-hydroxy triptolide with succinic anhydride to obtain the compound of formula II, or the step of phosphoric esterifying the second lead compound 12-β-thiocyano-13-α-hydroxy triptolide with $POCl_3$, PCl_3, phosphonate halide, phosphate, phosphite halide, or phosphite to obtain the compound of formula II.

The present invention provides a method for preparation of the compound of formula IIIa and IIIb, comprising the step of reacting the second lead compound 12-β-thiocyano-13-α-hydroxy triptolide with $POCl_3$ or other phosphonate halide, phosphate, phosphite and the like, or the step of reacting the first lead compound triptolide with $POCl_3$ or other phosphonate halide, phosphite halide and the like.

The present invention provides Use of the compounds of the present invention in the preparation of medicament as immunosuppressive agent or anti-inflammatory agent. In an embodiment, the immunosuppressive agent or anti-inflammatory agent is used in the

treatment of diseases associated with growth of lymphocytes T and B cells; production of cytokines such as IL-1, IL-2, IL-6, and iNOS; and production of Cox-2. In another embodiment, the diseases are autoimmune deficiency diseases and inflammatory diseases. In another embodiment, the diseases are selected from the group consisted of rheumatoid arthritis, asthma, systemic lupus erythematosus, psoriasis, multiple sclerosis, atherosclerosis, type I diabetes, and nephritis.

Another objective of the present invention is to provide molecular biological evidence of immunosuppressive activity of these novel highly water-soluble derivatives of triptolide (Example 8, 9). It has been demonstrated these novel derivatives of triptolide significantly inhibit the production of cytokines such as IL-1, IL-2, IL-6, iNOS and Cox-2.

A further objective of the present invention is to provide animal experiment evidence of low toxicity of these novel highly water-soluble derivatives of triptolide (Example 10). The water-soluble derivatives of triptolide prepared from T-SCN according to present invention have a significantly decreased toxicity. For example, the toxicity of sodium 12-β-thiocyano-triptolide-13-β-14-α-phosphate with a LD_{50} of 126 mg/kg body weight is much lower than that of triptolide which has a LD_{50} of 0.85 mg/kg body weight, while the former has a quite high immunosuppressive activity.

A still another objective of the present invention is to provide animal experiment evidence of treating autoimmune deficiency diseases with these novel highly water-soluble derivatives of triptolide. For example, the experiment of effect on DNCB-induced delayed hypersensitivity reaction of mice (Example 11) and anti-inflammatory test with cotton ball granulation in rat (Example 12) have demonstrated all of the novel WDY series derivatives of triptolide have significant immunosuppressive and anti-inflammatory activities.

Description of the Figures

Fig 1. Effect of water-soluble derivatives of triptolide according to the present invention on IL-2 (medium: blank control; PHA: lipopolysaccharide, CsA: Cyclosporine A).

Fig 2. Effect of water-soluble derivatives of triptolide according to the present invention on IL-1, IL-6 and iNOS (medium: blank control; PHA: lipopolysaccharide, CsA: Cyclosporine A).

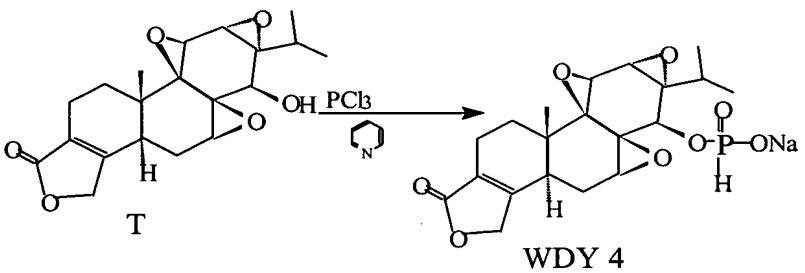
Fig 3. Effect of water-soluble derivatives of triptolide according to the present invention on PGE_2 content of HT-29 cell (pg/ml) (control = 22.93 pg/ml, indomethacin (indo) = 1.3 pg/ml, WDY 4 = 4.22 pg/ml, WDY 7 = 4.97 pg/ml).

Fig 4. The results of the effect of WDY 6 on contralateral paw.

Fig 5. The results of the effect of WDY 7 on contralateral paw.

Fig 6. The results of the effect of orally administrating WDY7 on the thickness of left paw.

Example 4. Preparation of sodium triptolide-14-β-phosphite (WDY 4)



T WDY 4

Under the protection of nitrogen atmosphere, 200 mg triptolide (0.556 mmol) and 20 ml pyridine were added into a three-necked bottle of 25ml, whereafter 0.10 ml PCl_3 (1.149 mmol) was slowly dropwise added. Upon feeding, nitrogen gas was stopped to enter the three-necked bottle. After reacting for 1 hour under sealing, the reaction bottle was cooled down in ice bath. Then saturated aqueous $NaHCO_3$ was slowly added to the reaction mixture to conduct hydrolytic reaction and neutralize to pH=9. The solvent in the mixture was vaporized to dryness under reduced pressure, and the residue was dissolved in chloroform/methanol (5/2) and the inorganic salts therein were removed. TLC detection was conducted by developing with n-butanol: water: glacial acetic acid (4:1:1), and major point was product WDY4 comprising a little other by-products. The product WDY4 was purified by H silica gel column chromatography using chloroform/methanol (5/2). The product was detected by TLC. The WDY4 was pooled, concentrated under reduced pressure to remove the solvent, dissolved in eluent and precipitated with ether to obtain purified product (yield 70 mg, 50%). R_f=0.46. Purple-red in developing reagent (Kedd's reagent). Water solubility >100 mg/ml.

IR(KBr)cm^{-1}: 3424, 2965, 2365, 1750, 1627, 1226, 1028, 972。

^{1}HNMR,δppm: 0.74(3H, d, J=7.2Hz, 16-CH_3), 0.90(3H, d, J= 6.8Hz, 17-CH_3), 0.94(3H, s, 18- CH_3), 1.25(1H, m, 1-αH), 1.32(1H, m, 1-βH), 1.80(1H, t , J=14.2Hz, 6- βH), 1.94(1H, m, 2-H), 2.10(1H, m, 2- H), 2.20(1H, m, 6-αH), 2.33(1H, m, 15- H), 2.59(1H, m, 5-H), 3.29(1H, m, 7-H), 3.52(1H, d, J=3.2Hz, 11-H), 3.82(1H, d, J=3.2Hz, 12-H), 4.03(1H, d, J=12.4Hz, 14-H), 4.83(2H, m, 19-H), 6.7(1H, d, J=595.6Hz, P-H).

^{13}CNMR, δppm: 13.9(18-C), 16.7(2-C), 17.0(16-C), 17.5(17-C), 22.8(6-C), 26.3(15-C), 29.2(1-C), 35.3(10-C), 40.1(5-C), 54. 3(12-C), 54.9(11-C), 60.4(7-C), 70.3(19-C), 73.5 (C-14, d, J=22.8Hz), 123.2(3-C), 162.5(4-C), 173.2(20-C), 63.5, 64.5, 64.6(8-C, 9-C, 13-C).

^{31}PNMR, δppm: 1.34(d, J_{P-H}=599Hz) 。

II

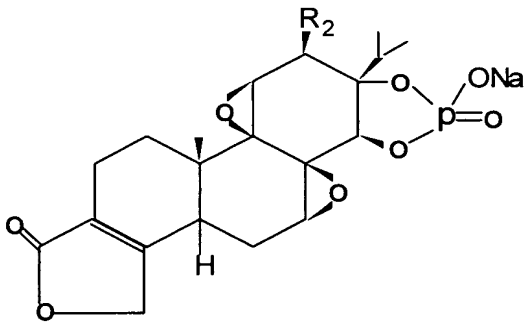
wherein R_1 is H, alkyl having 1-4 carbon atom(s), -C(=O)$(CH_2)_nCO_2$H-, wherein n is an integer of 1-4, or

phosphate (), or phosphite (),

wherein X_1 and X_2 are Na, K, or NH_4; and R_2 is H, -SCN, -Cl or -Br.

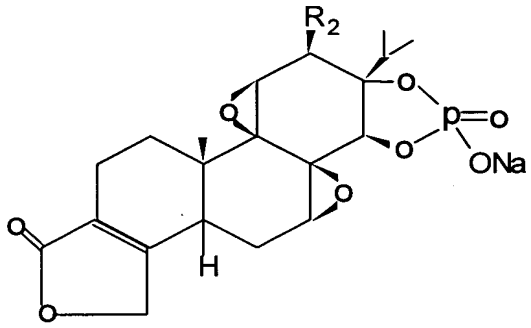
4. A method for preparation of the compound according to claim 3, comprising the step of esterifying ~~or phosphoric esterifying~~ the second lead compound 12-β-thiocyano-13-α-hydroxy triptolide with succinic anhydride to obtain the compound of formula II, or the step of phosphoric esterifying the second lead compound 12-β-thiocyano-13-α-hydroxy triptolide with $POCl_3$, PCl_3, phosphonate halide, phosphate, phosphite halide, or phosphite to obtain the compound of formula II.

5. A triptolide derivative of formula IIIa or IIIb



IIIa

or



IIIb

Wherein R_2 is H, SCN, Cl or Br.

6. A method for preparation of the compound according to claim 5, comprising

the step of reacting the second lead compound 12-β-thiocyano-13-α-hydroxy triptolide with $POCl_3$ or other phosphonate halide, phosphate, phosphite and the like, or

the step of reacting the first lead compound triptolide with $POCl_3$ or other phosphonate halide, phosphite halide and the like.

7. Use of the compounds according to any one of claims 1, 3, and 5 in the preparation of medicament as immunosuppressive agent or anti-inflammatory agent.